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Bacterial community changes in a glacial-fed Tibetan lake are correlated with glacial melting

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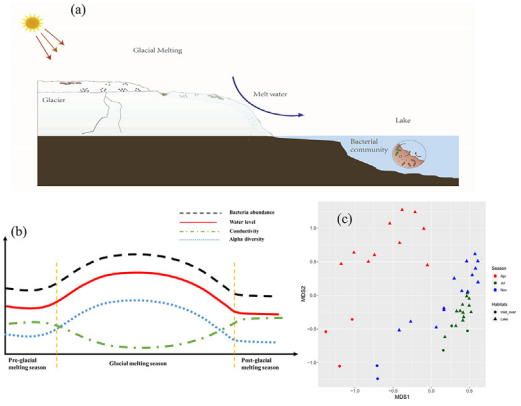
HIGHLIGHTS

- Bacterial communities exhibited obvious seasonal trends and significantly differed among the seasons.
- Bacterial communities changed along the gradient of conductivity concentration.
- The bacterial community composition, diversity and abundance were intensively affected by seasonal glacial melting.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate change-induced glacial melting is a global phenomenon. The effects of climate change-induced melting on the microbial ecology in different glacial-fed aquatic systems have been well illuminated, but the resolution of seasonal dynamics was still limited. Here, we studied bacterial community composition and diversity in a glacial-fed Tibetan lake, Lake Ranwu, to elucidate how glacial-fed aquatic ecosystems respond to the seasonal glacial melting. Obvious seasonal variations of bacterial dominant groups were found in Lake Ranwu and inlet rivers. In April, the majority of OTUs belonged to the Bacteroidetes, Actinobacteria and Proteobacteria. The Proteobacteria increased to the most abundant phylum in July and November, while the Bacteroidetes and Actinobacteria decreased about 50% over seasons. Most key discriminant taxa of each season's community strongly associated with specific environmental variables, suggesting their adaptation to seasonal environments. Bacterial alpha diversity varied among seasons and exhibited strongly negative correlations with conductivity. Conductivity was the major driving force in determining the seasonal variation of bacterial community composition. Fluctuated conductivity was one of the consequences of seasonal melting of glaciers. This study offered evidence for the unique seasonal dynamics pattern of bacterial communities responding to glacial melting.

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Moreover, this study may provide a reference for assessing the long-term effects of glacial retreat on glacial-fed aquatic ecosystems.

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1. Introduction

Glaciers are sensitive indicators of climate change (Moore et al., 2009). Continuing glacial melting is one of the most prominent signals of global climate change (Stocker et al., 2013). Glacial melting increases the meltwater immediately, but decreases the amount of meltwater in the long term with the coverage of glacial area decreasing. Shifts in the water supply influence the physicochemical characteristics of downstream ecosystems, which subsequently effect on microbial communities, leading to the total loss of microbial biodiversity in glacier-fed lakes/streams (Brown et al., 2007; Milner et al., 2009; Solomina et al., 2015; Milner et al., 2017). Bacterial diversities in glacial-fed lakes are significantly decreased with turbidity owing to the dilution of the lake water by the melting of glaciers (Peter and Sommaruga, 2016). Wilhelm et al. (2013) have found that the shrinking of glaciers increased bacterial alpha diversity but reduced bacterial beta diversity in glacial melt-fed streams across 26 alpine glaciers (Wilhelm et al., 2013). These studies make clear that glacial melting drives the spatial variability of bacterial community composition and diversity.

Glacial melting has a profound influence on glacial-fed aquatic systems over a timescale of days to millennia (Moore et al., 2009). Therefore, the response of glacial-fed aquatic ecosystems to glacial melting-induced environmental changes will have varied through time. At the seasonal timescale, glaciers melt in summer and increase the meltwater quantities. During the melting season, as meltwater quantity increase, dilution of dissolved salt concentrations is observed (Anderson, 2007; Milner et al., 2009). Seasonal shifts in the hydrological regime in the glacial-fed aquatic systems will alter the physicochemical properties to which microbial communities have adapted. However, how microbial communities in the glacial-fed aquatic systems respond to seasonal glacier-induced environmental changes remains unclear. Thus, time-series studies of the current melting of glaciers could be beneficial research strategies to understand how the long term glacial melting affects the ecosystem.

Previous studies suggest that bacterial communities in the glacial-fed aquatic systems are assembled by deterministic processes such as environmental filtering (Wilhelm et al., 2013; Milner et al., 2017). The concept of environmental filtering proposes that changes in species abundances and diversity patterns along environmental gradients, such as conductivity, temperature, and chlorophyll *a* (Chl *a*) concentration, based on their traits and adaptations to prevailing environmental conditions (Lebrija-Trejos et al., 2010; Lindstrom and Langenheder, 2012; Sommer et al., 2014). Glacier-fed lakes/streams are examples of harsh environments and associate with low temperature, nutrient deficiency and low taxonomic richness (Jacobsen and Dangles, 2012; Jacobsen et al., 2012). It may therefore be that harsh environments imposed strong environmental filtering on bacterial communities in the glacial-fed aquatic systems.

The Tibetan Plateau contains the largest area of glaciers outside the polar regions with a total area of 49,873 km^2 (Immerzeel et al., 2010; Yao et al., 2010). The Tibetan Plateau has the largest lake group in the Asian continent, where the lakes respond sensitively to climate change. Glaciers are continuously melting, resulting in expanding glacial-fed lake area in this region (Xin et al., 2009; Yang et al., 2008; Yao et al., 2007). In the past few decades, climate warming-induced glacial melting has strongly affected the environment and aquatic ecosystems on the Tibetan Plateau (Chen et al., 2013; Yao, 2010). The Tibetan Plateau serves as the main water storage reservoir for South and East Asia (Immerzeel et al., 2010), it is especially important to understand the influence of seasonal glacial melting on microbial communities in downstream glacial-fed lakes in this region.

Here, we explored the seasonal variations in bacterial community composition and diversity in Lake Ranwu, which is located in the proximity of rapidly melting glaciers in the southeastern Plateau. This study aims to obtain an in-depth understanding of seasonal changes in glacial-fed bacterial communities and their response to glacial melting. Studies of the geomorphology and remote sensing data of the Lake Ranwu catchment show that the glacial coverage decreased dramatically and the lake area expanded annually in the last 25 years (Yao et al., 2010). Because the high discharge during summer ablation resulted in an increase input of microbes into glacial melt-fed lakes and low dissolved salt concentrations in downstream, we hypothesized that bacterial diversity increased with the discharge of glacial melting in Lake Ranwu. The environmental harshness of a specific lake site is highly dependent on its distance from the glacier and the size of the glacier meltwater discharge (Moore et al., 2009). Given that many glacial-fed lakes generally characterized by low nutrients, temperature and productivity, we postulated that environmental filtering played a key role in shaping bacterial community composition and diversity in Lake Ranwu.

2. Materials and methods

2.1. Study area

Lake Ranwu (96°30′~97°10′ E, 29°00′~29°50′ N, 3920 m above sea level) is a glacial-fed lake located in the southeast of the Tibetan Plateau (Fig. 1). The lake is braided channel shape, with a total length of 29 km and an average width of about 800 m (Xin et al., 2009). Lake Ranwu is divided into three major areas: the upper, intermediate, and downstream. The Quchihe (QCH), Zhongkongnongba (ZKNB) and Renongba (RNB) rivers are the primary sources of water to Lake Ranwu (Fig. 1). The upper area is located close to the glacial river QCH and the intermediate area is located close to the ZKNB river. Finally, water from the RNB river and from the intermediate area mixes in the downstream area. The source of the QCH River is melt water mainly from the Yanong, Xirinongpu and Zuoqiupu Glaciers (Ju et al., 2015). The QCH River, as the largest inflow and a glacial melt river, has an important impact on the water level and hydrological characteristics of Lake Ranwu (Ju et al., 2015).

2.2. Field sampling and physiochemical analyses

Sampling was carried out in spring (14th–15th, April, the pre-glacial melting season), summer (14th–15th, July, the glacial melting season), and autumn (14th–15th, November, the post-glacial melting season) in 2012. As shown in Fig. 1, water samples were collected from the upper (R1), intermediate (R2), and downstream (R3) areas of Lake Ranwu and three inlet rivers (QCH, ZKNB and RNB). Lake samples were collected from the different depths (0.5, 5, 10, 15, 20 and 25 m) of the water column, and river samples were collected from surface (0.5 m) (Table S1).

The concentrations of dissolved oxygen (DO) and chlorophyll *a* (Chl *a*), pH, conductivity (Cond) and water temperature (T) were monitored in situ with a multi-probe Water Quality Sonde (Hydrolab DS5, Hach, USA). For assessing the bacterial community composition, approximately 1 L water were collected with a Schindler sampler and filtered onto a 0.22 μm polycarbonate membrane (Millipore, USA).

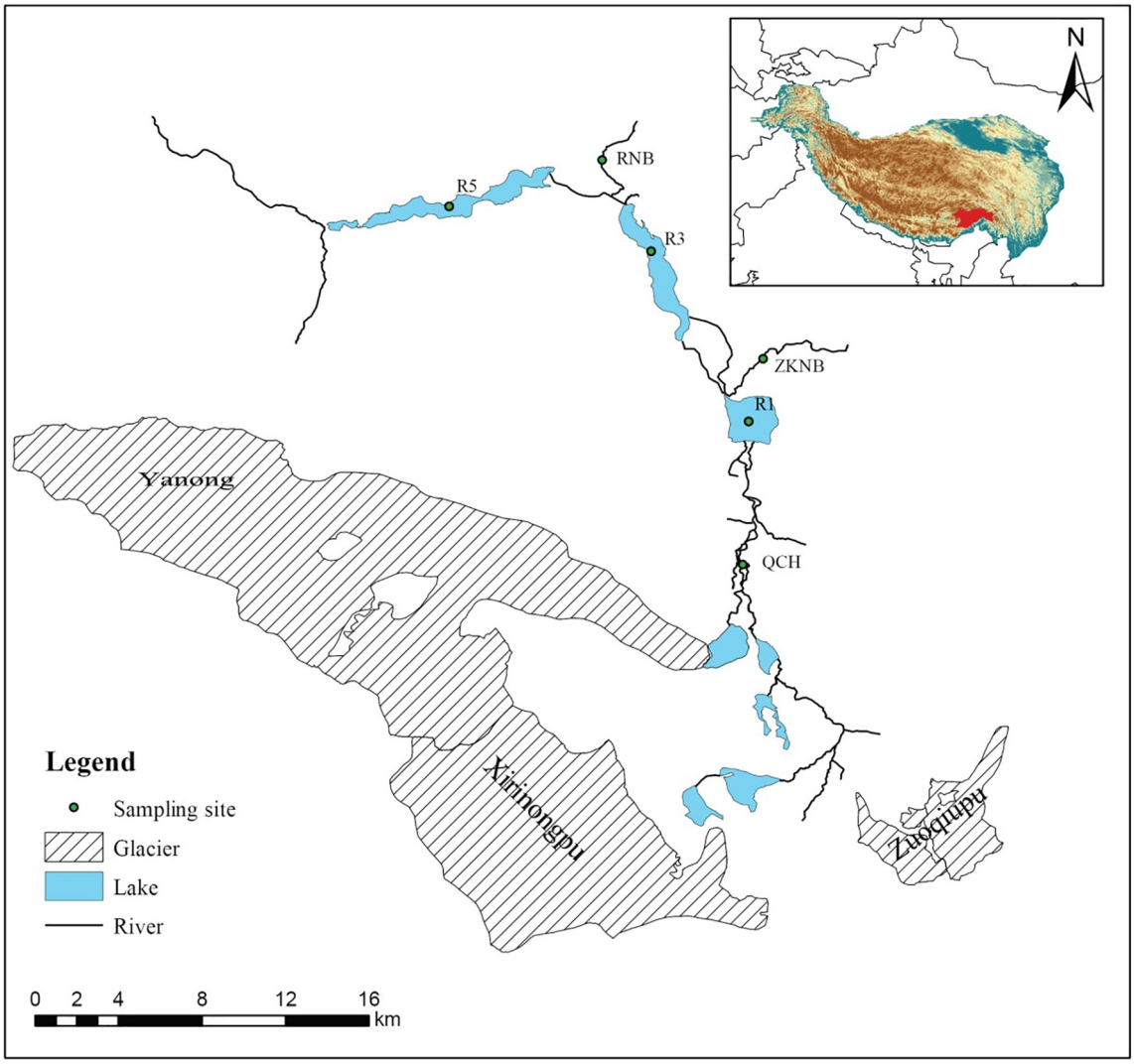

Fig. 1. Sampling sites of Ranwu catchment with the Yanong, Xirinongpu and Zuoqiupu glaciers, the upper lake (R1), the intermediate lake (R3), the downstream Lake (R5) and three inlet rivers (QCH, ZNB, and RNB).

For bacterial enumeration, duplicate 2 mL aliquots from each sample were fixed immediately with glutaraldehyde (1.5% final concentration) on location, stored in the dark at a temperature of 4 °C, and analyzed within 3 months. Bacterial abundance was analyzed using a flow cytometer (Beckman Coulter, Epics Altra II) equipped with an external quantitative sample injector (Harvard Apparatus PHD 2000) using SYBR Green I (Molecular Probes) nucleic acid stain as described previously (Jiao et al., 2005).

100 mL water sample was filtered through 0.45 μm cellulose membranes (Millipore, Billerica, MA, USA) to measure dissolved organic carbon (DOC) and total nitrogen (TN) as previously reported (Liu et al., 2014) using 20-mL glass bottles (1% HCl leached, deionized water rinsed, and 450 °C >3 h combusted) from each sample, and frozen at −20 °C until analysis. The DOC and TN concentrations were measured with a TOC-Vcph (Shimadzu Corp., Japan) following the standard methods.

2.3. DNA extraction

Microbial genomic DNA was extracted with a Fast DNA®SPIN Kit for Soil (MP Biomedicals, Santa, CA), following the manufacturer's instructions. The raw DNA was purified using a 1% (w/v) agarose gel. DNA bands were excised from the gel, extracted using an Agarose Gel DNA purification kit (TaKaRa, Japan). Then, the concentration and purity of the genomic DNA extracts were quantified with a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted DNA was stored at −80 °C until amplification.

2.4. Bacterial 16S rRNA amplification and Illumina MiSeq sequencing

The V4 region of the bacterial 16S rRNA genes was amplified in triplicate for each sample with the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) combined with Illumina adapter sequences, a pad and a linker of two bases, as well as barcodes on the reverse primers (Caporaso et al., 2012). Positive PCR products were confirmed using agarose gel electrophoresis. PCR products from triplicate reactions were combined and quantified with PicoGreen. PCR products from samples to be sequenced in the same MiSeq run were pooled at equal molality. The pooled mixture was purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences, Germantown, MD, USA) and re-quantified with PicoGreen. Sample libraries were generated from purified PCR products. The MiSeq 500 cycles kit was used for 2 × 250 bp paired-ends sequencing on a MiSeq machine (Illumina, San Diego, CA). The 16S rRNA sequences were submitted to NCBI SRA database (BioProject accession number: PRJNA342071).

2.5. Processing of Illumina sequencing data

The overlapped paired-end sequences from Miseq were assembled with FLASH (Magoč and Salzberg, 2011) and poorly overlapped and poor quality sequences were filtered out before de-multiplexing based on barcodes. We processed the sequences mainly using QIIME pipeline (v1.8.0) (Caporaso et al., 2010). Briefly, bacterial reads were truncated at any site containing more than three consecutive bases receiving a quality score < 20, and any read containing one or more ambiguous base calls was deleted. In addition, the truncated reads <75% (of the raw read length) consecutive high-quality base calls were discarded. Then, the sequences were clustered into OTUs at 97% pairwise identity with the uclust algorithm (Edgar, 2010). Bacterial alpha diversity estimates, including Shannon diversity and Chao1-richness, were calculated by using the QIIME script 'alpha_diversity.py'. Representative sequences from each OTU were aligned using PyNAST (Caporaso et al., 2010). The taxonomic identity of each representative sequence was determined using the reference database SILVA (version 132 NR) (Pruesse et al., 2007). After taxonomies had been assigned, OTUs that were affiliated with chloroplast, archaeal and unclassified sequences were removed.

2.6. Statistical analysis

To better reveal changes of bacterial communities and their response to glacial melting, samples were combined to different groups according to the sampling time (season) or the sampling environments (habitats). Permutational multivariate analysis of variance (MANOVA) was conducted to quantitatively evaluate the contribution of season (April or July or November) and habitats (inlet river and lake) to bacterial community variation using the 'adonis' function (Anderson, 2001). One-way analysis of variance (ANOVA) was used to compare the differences in environmental variables and bacterial alpha diversity. The significantly discriminant taxa in each major cluster (seasonal groups) were determined using the LEfSe method (Segata et al., 2011). Non-metric multidimensional scaling analysis based on weighted UniFrac distance, was applied to visualize the overall differences of bacterial community composition between samples (Dixon, 2003). Analysis of similarity (ANOSIM) was performed to evaluate the over-all differences in each seasonal bacterial community composition based on weighted UniFrac distance metrics (Clarke, 1993). A distance-based multivariate linear model (DISTLM) based on weighted UniFrac distance was performed using the DISTLM_forward3 program to determine which environmental variables significantly explain the observed similarity among the samples (McArdle and Anderson, 2001).

We applied aggregated boosted tree (ABT) analysis (De'Ath, 2007) with the "gbm" and "dismo" package in R to quantitatively and visually evaluate the relative influence of environmental variables on bacterial alpha diversity. For an ABT analysis, it can deal with many types of response variables (numeric, categorical and censored) and environmental variables (numeric, categorical). Furthermore, it can quantitatively and visually evaluate the relative influence of environmental variables on each diversity estimate (De'Ath, 2007).

We implemented the SEGMENTED method to evaluate the environmental variables change point as a threshold by integrating linear and generalized linear regression models with the "segmented" R package (Muggeo, 2008). The change point found using SEGMENTED is the mean with its standard error of potential change point observed in a range of the key environmental variables (Vanacker et al., 2016). We also applied the Davies test (Davies, 1987) to check whether there was a significant difference in the slope. All statistical analyses were performed with the statistical program R unless otherwise indicated (R Core Team, 2014).

3. Results

3.1. Water level and environmental characteristics

The water level of Lake Ranwu was impacted mainly by the glacial melting and varied among seasons (Fig. S1). The water level showed a progressive increase from April to June, obtained a stable higher water level during June to August, and then displayed a decreasing pattern from September. The seasonal changes of water level in Lake Ranwu were consistent with QCH (linear regression, $R^2 = 0.966$, $P < 0.001$) and ZKNB rivers ($R^2 = 0.949$, $P < 0.001$).

Conductivity in inlet rivers and Lake Ranwu exhibited significant differences among seasons (ANOVA, $P < 0.001$; Table 1 and Table S1). Conductivity was significantly highest (River 151 ± 52 μS cm^{-1} vs. Lake 140 ± 2 μS cm^{-1}) in April during the pre-glacial melting season, and lowest (River 85 ± 31 μS cm^{-1} vs. Lake 86 ± 5 μS cm^{-1}) in July when melt water dominated the inflows. Conductivity varied among the three seasons and showed marginally significant correlation with water level (Pearson's correlation, $r = -0.667$, $P = 0.069$).

Bacterial abundances in inlet rivers and lake synchronously exhibited seasonal differences, with the highest abundances in July and the lowest in April (Fig. 2a and b). Bacterial abundance were related negatively with conductivity ($r = -0.493$, $P < 0.001$) and positively with water temperature ($r = 0.459$, $P = 0.002$) (Fig. S2).

3.2. Taxon-specific seasonal patterns

After quality control, a total of 2,003,521 high quality sequences were obtained, of which there were 21,192 to 85,431 (mean = 44,522 ± 11,110, n = 45) sequences per sample. To avoid sample size-based artifacts, we used a randomly selected subset of 21,100 sequences (corresponding to the smallest sequencing effort for any of the samples) per sample to calculate the community diversities and distances between samples.

The taxonomic classification illustrated that the majority of OTUs belonged to the Bacteroidetes (31%), Actinobacteria (26%) and

Table 1
Rivers and lakes water sample geochemical parameters in April, July and November. The data represent the mean values of samples in each month with standard deviation. Results of statistical analyses of differences between groups are shown using one-way analysis of variance (ANOVA), with $P < 0.05$ considered significant. Different letters indicate those means which are significantly different.

Date	Sample sites	T (°C)	pH	Cond (μS cm^{-1})	DO (mg L^{-1})	Chl *a* (μg L^{-1})	DOC (mg L^{-1})	TN (mg L^{-1})	Sample number
2012/04	April-R	2.8 ± 0.9 cd	9.2 ± 0.2a	150.8 ± 51.9ab	10.49 ± 0.3a	86.97 ± 30.13a	0.326 ± 0.091bc	0.185 ± 0.080b	3
2012/04	April-L	4.8 ± 1.0b	8.1 ± 0.1c	140.2 ± 2.3b	7.06 ± 0.2b	0.28 ± 0.08c	0.357 ± 0.085b	0.117 ± 0.011c	10
2012/07	July-R	7.9 ± 4.1a	7.9 ± 0.4c	85.3 ± 30.9d	7.11 ± 0.9b	0.37 ± 0.42c	0.327 ± 0.084bc	0.128 ± 0.022bc	3
2012/07	July-L	8.1 ± 2.0a	8.4 ± 0.1b	85.9 ± 5.4d	6.81 ± 0.3c	0.76 ± 0.35b	0.273 ± 0.073c	0.123 ± 0.018c	13
2012/11	November-R	1.2 ± 1.6d	8.6 ± 0.1b	169.9 ± 51.1a	10.83 ± 0.9a	91.30 ± 22.20a	3.281 ± 0.284a	0.487 ± 0.033a	2
2012/11	November-L	3.3 ± 0.6c	8.8 ± 0.3ab	101.1 ± 3.5c	7.22 ± 0.2b	0.37 ± 0.24c	2.308 ± 1.501a	0.259 ± 0.105b	14
	ANOVA (F)	17.64	19.79	23.65	92.75	116.80	12.64	17.35	
	ANOVA (*P*)	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	

T: temperature, Cond: conductivity, DO: dissolved oxygen, Chl *a*: chlorophyll *a*, DOC: dissolved organic carbon, TN: total nitrogen.

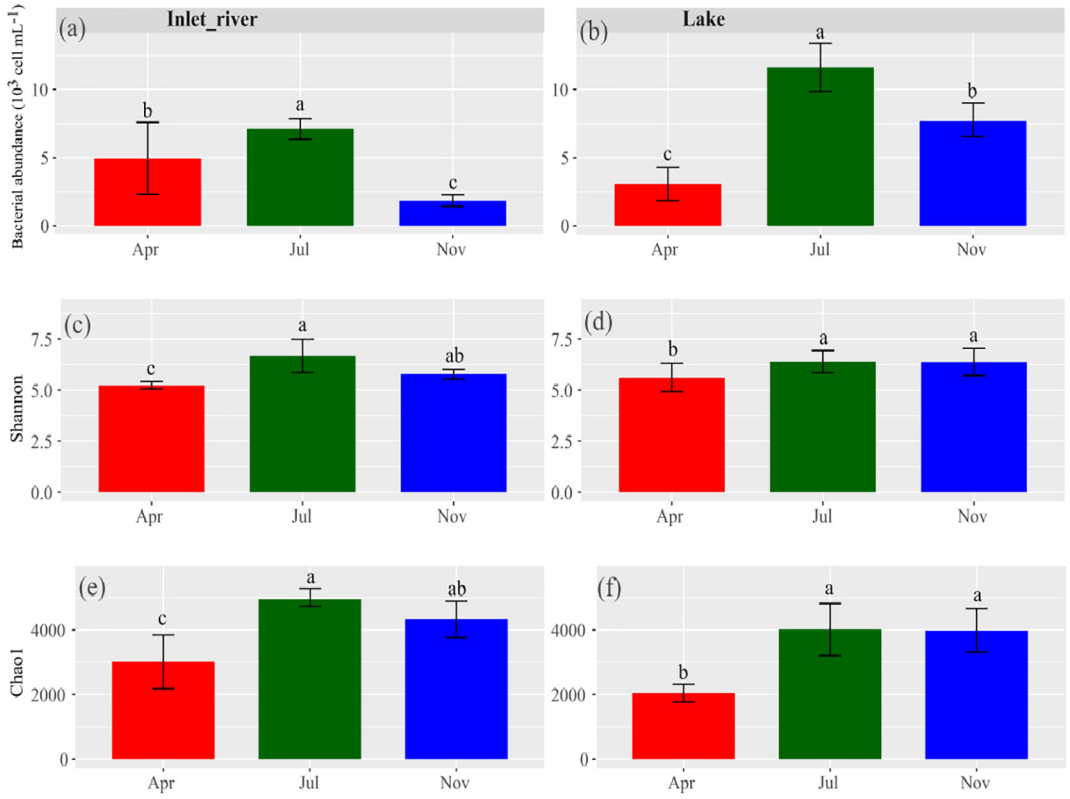

Fig. 2. Seasonal pattern of bacterial abundance and the bacterial alpha diversity indices. Seasonal trends of bacterial abundance, Shannon diversity, and Chao1-richness in the inlet rivers (a, c, and e) and Lake Ranwu (b, d, and f). Different lowercase letters indicate significant differences among seasons. Means were compared using one-way analysis of variance (ANOVA), with $P < 0.05$ considered significant.

Proteobacteria (25%) were observed in inlet rivers and lake in April (Fig. 3 and Fig. S3). However, the Proteobacteria increased to the most abundant phylum in July and November, represented 51% and 43% of the total sequence, respectively. The Bacteroidetes and Actinobacteria decreased over seasons, accounted 15% and 13% of total OTUs respectively in July and both 15% in November. Several less abundant phyla, such as the Firmicutes (3%), were primarily found in lake in April (Fig. S3).

The LEfSe algorithm revealed that 48 predominant bacterial taxa were statistically significantly discriminative either for April (n = 19), July (n = 13) or November (n = 17) bacterial communities (Fig. S4). At the phylum level, the Firmicutes and Cyanobacteria (related to the genus *Leptolyngbya*) were characteristic for April and November bacterial communities, respectively. The Flavobacteria (related to the genus *Flavobacterium*), Bacteroidia (related to the genus *Bacteroides*), Actinobacteria (related to the family *Microbacteriaceae*), Planctomycetes (related to the family *Isosphaeraceae*) and Betaproteobacteria (related to the genus *Polynucleobacter*) were further identified as April communities. The Bacteroidetes (related to the families *Sphingobacteriaceae*, *Cytophagaceae* and *Cyclobacteriaceae*), Alphaproteobacteria (related to the families *Acetobacteraceae*, *Rhodospirillaceae* and *Erythrobacteraceae*) and Betaproteobacteria (related to the order SBla14 and the family *Comamonadaceae*) were more abundant in July communities. The Bacteroidetes (related to the genus *Spirosoma*), Actinobacteria (related to the order Gaiellales and the family *Intrasporangiaceae*), Planctomycetes (related to the families *Pirellulaceae* and *Planctomycetaceae*), Alphaproteobacteria (related to the family *Sphingomonadaceae* and the genera *Phenylobacterium* and *Rhodobacter*) and Betaproteobacteria (related to the order *Rhodocyclales*) were distinctive features of the November communities.

The highly abundant discriminant taxa (at the family or genus levels) of each season could be well clustered using their Pearson correlation coefficients with environmental variables (Fig. 4a). The discriminant taxa in April exhibited generally positive correlations with conductivity. Contrarily, discriminant taxa in July and November were negatively correlated with conductivity. Positive correlations with temperature were found only in July bacterial communities. Several discriminant taxa (for example, *Phenylobacterium* and *Rhodobacter*) in November showed significant correlations with pH ($P < 0.001$ and $P = 0.035$, respectively) and DOC ($P = 0.012$ and $P = 0.001$, respectively).

3.3. Bacterial alpha diversity seasonal patterns

Bacterial alpha diversity indices, including Shannon diversity (Fig. 2c and d) and Chao1-richness (Fig. 2e and f), significantly differed among the seasons (ANOVA, $P < 0.05$ in all cases) in both the inlet rivers and lake, with the highest mean values in July and the lowest mean values in April. According to ABT model, conductivity was the most important variable in explaining the variations in Shannon diversity (relative influence of 30%, Fig. S5a) and Chao1-richness (37%, Fig. S5b). Both of Shannon diversity and Chao1-richness exhibited strongly negative correlations with conductivity (Pearson's correlation, $r = -0.425$, $P = 0.004$, and $r = -0.374$, $P = 0.011$, respectively; Table 2).

There was an apparent shift of bacterial alpha diversity along with conductivity gradient (Fig. S6). The SEGMENTED method identified that the distinct change points of conductivity were approximately 126 ± 7 and 121 ± 8 μS cm^{-1} for Shannon and Chao1-richness (Fig. S6 and Table S2). The Davies test showed there was a significant difference in change point for Shannon ($P = 0.037$) and Chao1-richness ($P = 0.036$).

3.4. Bacterial community composition seasonal patterns

Non-metric multidimensional scaling revealed there was a clear seasonal pattern of bacterial community composition in both inlet rivers

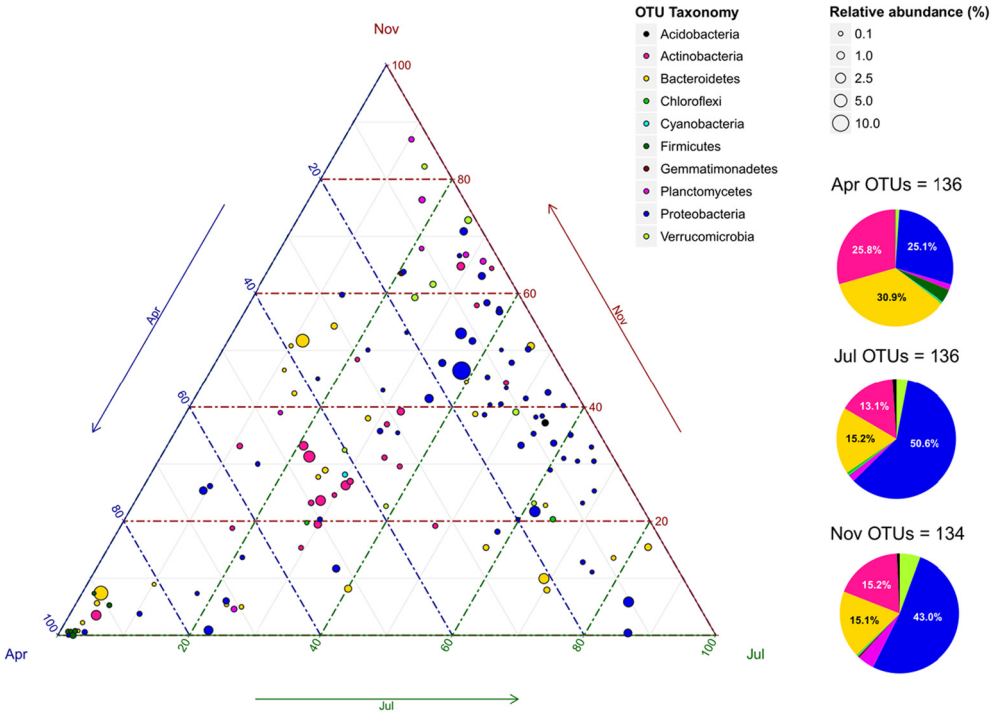

Fig. 3. Ternary plot showing the seasonal distribution and relative abundance of OTUs (relative abundance >0.5% in at least one sample). The size of the circle indicates the relative abundance of each OTU in total samples. Apr, April (spring, the pre-glacial melting season, 2012); Jul, July (summer, the glacial melting season, 2012) and Nov, November (autumn, the post-glacial melting season, 2012).

and lake (Fig. 4b). Bacterial community compositions in inlet rivers and lake significantly varied among seasons based on an analysis of similarity (ANOSIM) (Table S3, global $r = 0.558$, $P = 0.001$). Permutational MANOVA revealed that season (April or July or November) accounted for 27% ($P < 0.001$) of the total variation in bacterial community composition, whereas habitat (inlet river or lake) constrained 14% ($P = 0.017$), and the interaction between season and habitat was not significant ($P = 0.575$, Table S4). The seasonal variation of bacterial community composition was more pronounced than habitats variation in Lake Ranwu watershed (Table S4).

To better assess the effects of environmental variables on the variations of the bacterial communities in different seasons, we used the DistLM to minimize autocorrelation between the environmental variables (McArdle and Anderson, 2001). Of all the environmental variables examined, conductivity was correlated mostly with total bacterial community and explained 30% of the total variation of the bacterial community composition (Table 3). In addition, three other significant variables (Chl *a*, TN and temperature, $P < 0.05$ in all cases) explained 21% of the total variation of the bacterial community composition.

4. Discussion

In the current study, our results showed that seasonal glacial meltwater dynamics had a strong influence on the bacterial community composition and diversity as well as bacterial abundance, in glacial melt-fed Lake Ranwu.

4.1. Bacterial taxa indicating seasonal glacial melting influence

Following glacial melting, the seasonal changes in physiochemical conditions have played critical roles in influencing the distribution of bacterial taxa in the Lake Ranwu watershed. Summer glacial melting led to distinct physicochemical water characteristics, resulting in the diversification of better adapted taxa. LEfSe analysis indicated that the bacterial communities in different seasons could be distinguished based on the abundance of several key discriminant taxa (Fig. S4). These key taxa display different ecological strategies across environments (Hodson et al., 2008; Knelman et al., 2012), and are strongly associated with specific environmental variables (Fig. 4a). For instance, the discriminant taxa belonging to the genus *Natronobacillus* (affiliated to Firmicutes) were positively correlated with conductivity in April. Members of the *Natronobacillus* were alkaliphilic and salt-tolerant natronophiles, and have been found in a variety of aquatic and terrestrial habitats (Galperin, 2013). Flavobacteria (related to the genus *Flavobacterium*), which are known for their abilities to rapidly exploit bioavailable organic matter (Eiler and Bertilsson, 2007), were significantly positively correlated with Chl *a*. The Alphaproteobacterial genus *Rhodobacter* includes species which possess an extensive range of metabolic capabilities such as photosynthesis, lithotrophy, and aerobic and anaerobic respiration (Haselkorn et al., 2001). These organisms are found in a number of varied freshwater or marine environments (Crump et al., 1999; Dang and Lovell, 2002; Héry et al., 2014). The relative abundances of *Rhodobacter* species were positively correlated with the concentrations of DOC, TN and temperature, which implied that nutrient and temperature appeared to be important factors in structuring the distribution of *Rhodobacter*. These results are in agreement with the notion that environmental filtering, that is adaption to local environmental conditions (e.g., Chl *a*, DOC, TN and temperature), may result in the species abundance seasonal patterns (De Wit and Bouvier, 2006).

4.2. Variation in alpha diversity of the bacterial community along the conductivity gradient

The rapid melting of glaciers is reported to have a significant impact on the biodiversity of glacial melt-fed aquatic ecosystems (Jacobsen and Dangles, 2012; Jacobsen et al., 2012). Our study indicated that bacterial alpha diversity sensitively responded to the seasonal variations induced by glacial melting, and showed significant correlations with conductivity. Conductivity is related to the total dissolved salt content of the

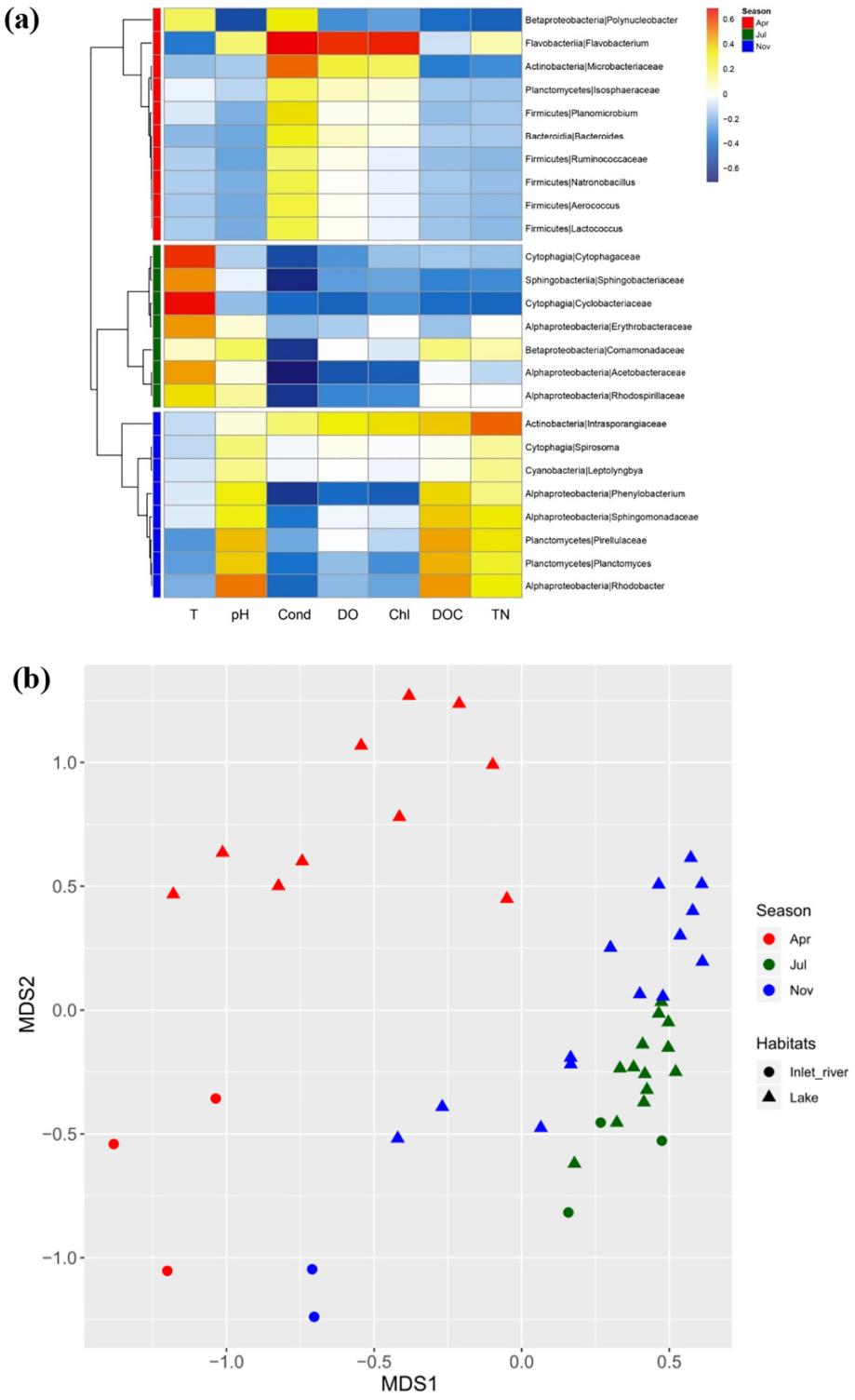

Fig. 4. (a) Heat map of Pearson correlations between environmental variables and highly abundant discriminant taxa of the three seasons. Colors represent the *r*-values of Pearson correlations between the relative abundances of the discriminant taxa and environmental parameters. (b) Non-metric multidimensional scaling (NMDS) ordination of the dissimilarity (weighted UniFrac distance) in bacterial community composition (stress = 0.055). T: temperature, Cond: conductivity, DO: dissolved oxygen, Chl: chlorophyll *a*, DOC: dissolved organic carbon, TN: total nitrogen.

water. Significant changes in conductivity can be a useful indicator that meltwater has entered glacial-fed aquatic systems. During the initial phase of glacial melting, the melt water flow usually produce high conductivity concentrations because of the accumulated salt and dust from the non-melting season. However, as the snow and ice melts, flow increases and the large amount of meltwater that is released acts to decrease the conductivity concentration during the glacial melting season.

Our ABT model analysis demonstrated that conductivity is the most important factor influencing the alpha diversity of the bacterial communities (Fig. S5). Similarly, Wilhelm et al. (2013) reports that conductivity is significantly related to the "true richness" of glacial melt-fed

Table 2
Linear regression analyses between environmental variables and bacterial alpha diversity.

	Shannon		Chao1	
	r	*P*	*r*	*P*
T	**0.314**	0.035	**0.299**	0.046
pH	0.009	0.951	0.188	0.217
Cond	**−0.425**	0.004	**−0.375**	0.011
DO	**−0.374**	0.011	−0.094	0.539
Chl *a*	**−0.336**	0.024	0.012	0.938
DOC	0.091	0.552	0.193	0.205
TN	0.052	0.735	0.268	0.075

Data in bold indicate significant correlations.

stream water communities (Wilhelm et al., 2013). The results of the SEGMENTED method revealed that there were obvious responses of bacterial alpha diversity for conductivity, and the change point threshold levels of conductivity were 126 ± 7 and 121 ± 8 μS cm^{-1} where peaked at the highest alpha diversity. The change point thresholds were between November (110 ± 27 μS cm^{-1}) and April (143 ± 22 μS cm^{-1}), but higher than in July (86 ± 12 μS cm^{-1}). All these features demonstrated that the responses of the bacterial alpha diversity to conductivity were controlled by seasonal glacial melting. Our findings also clearly indicated that low or high conductivity concentration might minimize bacterial alpha diversity, suggesting that bacterial communities may be most resistant to the influence of glacial melting at intermediate levels of conductivity concentration. This pattern was consistent with the intermediate disturbance hypothesis (Connell, 1978), which declares that bacterial diversity is expected to peak at intermediate levels of environmental disturbance. One possible reason was that decreased conductivity due to high glacial meltwater discharge is beneficial to freshwater bacterial communities, resulting in higher bacterial alpha diversity in July. As Wang et al. (2011) reported that bacterial diversity were relatively higher in low salinity than that in high salinity in Tibetan lakes (Wang et al., 2011). Another possible reason was that enhanced glacial meltwater may carry microorganisms storage in glacier to migrate downstream lake, thereby increasing glacial-fed lake bacterial alpha diversity (Wilhelm et al., 2013; Peter and Sommaruga, 2016).

Water temperature showed significant positive correlations with water bacterial alpha diversity and abundance as well (Table 2, Fig. S2). The lower temperatures in the pre-glacial melting season and the post-glacial melting season might limit bacterial growth by reduced bacteria substrate utilization capacity, leading to lower water bacterial alpha diversity and abundance in April and November in Lake Ranwu. This is in accordance with a few previous studies in which lower temperature were confirmed to impose stronger environmental filtering, decreasing the bacterial diversity and abundance in glacial-fed aquatic ecosystems (Freimann et al., 2013; Mindl et al., 2007).

Table 3
Distance-based multivariate linear model of bacterial community composition showing percentage of variation explained by environmental variables. (weighted UniFrac distance, 999 permutations).

Variable	Pseudo-F	*P*	Percent variation explained	Cumulative variation explained
Marginal tests				
T	3.591	**0.018**	0.08	
pH	2.200	0.069	0.05	
Cond	18.033	**0.001**	0.30	
DO	10.819	**0.001**	0.20	
Chl *a*	12.490	**0.001**	0.23	
DOC	2.151	**0.074**	0.05	
TN	1.508	0.170	0.03	
Sequential tests				
Cond	18.033	**0.001**	0.30	0.30
Chl *a*	7.955	**0.001**	0.11	0.41
TN	4.966	**0.001**	0.06	0.47
T	2.829	**0.013**	0.03	0.51
DO	1.534	0.163	0.02	0.53
pH	1.179	0.302	0.01	0.54
DOC	0.390	0.894	0.00	0.54

Marginal tests: each variable was analyzed individually (ignoring other variables); Sequential tests: forward selection of variables, where the percentage of variation explained by each variable added to the model was conditional on variables already in the model. Data in bold present significant correlations ($P < 0.05$).

4.3. Changes in bacterial community composition along the conductivity gradient

One of the consequences of seasonal melting of glaciers was the seasonal fluctuation of conductivity (Table 1 and Table S1). Conductivity was the most important variable controlling the seasonal dynamics of bacterial community composition in Lake Ranwu (Table 3). Several previous investigations document that conductivity is a key factor in shaping spatial distribution pattern of bacterial communities in glacial melt-fed stream (Freimann et al., 2013; Wilhelm et al., 2013). Compared to other glacial-fed streams, the observed range of conductivity fluctuated much larger (from 86 to 143 μS cm^{-1}) in this study. In an Ecuadorian glacial-fed stream, Jacobsen et al. (2010) reported that conductivity was between 11 and 17 μS cm^{-1} along the stream and 6 μS cm^{-1} close to the glacier (Jacobsen et al., 2010). In glacier-fed streams in Austria, Wilhelm et al. (2013) also reported that conductivity ranged from 3 to 83 μS cm^{-1} across 26 Alpine glaciers (Wilhelm et al., 2013). Conductivity is a measure of water conduction current, which is directly related to the total dissolved salt content of water. Aquatic microbes require a relatively constant concentration of the major dissolved ions in the water. Levels too high or too low may limit microbial survival, especially in freshwater environments. That may be the reason why the fluctuation of conductivity shows significant influence on the bacterial community composition in this study.

In addition conductivity, Chl *a* and TN were the important factors driving the seasonal variation of bacterial community composition in Lake Ranwu, consistent with studies in Alpines and Himalaya lakes (Battin et al., 2001; Liu et al., 2006). Temperature also had an important impact in bacterial community composition in the present study. Like it is reported to be significant determinant in bacterial/planktonic/invertebrate communities in glacial-fed environments (Freimann et al., 2013; Milner et al., 2009; Sommaruga, 2015). Overall, our results showed that the bacterial community composition in Lake Ranwu varied during different seasons, and is controlled by seasonal glacial melt-induced physicochemical water characteristics. These observed patterns might reflect the importance of local environments in structuring bacterial communities in Lake Ranwu.

5. Conclusion

In conclusion, this study investigated the bacterial community composition and diversity shifts as a consequence of seasonal changes in glacial melt in Lake Ranwu in the southeastern Tibetan Plateau. Our study demonstrated that the bacterial community composition and diversity in Lake Ranwu were affected by seasonal glacial melting. We found that conductivity played pivotal roles in maintaining bacterial community composition. During the summer melting season, bacterial abundances and alpha diversity increased as the amount of melt water increased. With climatic change-induced glacial retreat projected to continue, long-term studies and observations are vital to predict glacial-fed aquatic ecosystem trajectories regarding changes in microbial community and diversity.

Conflict of interests

The authors declare no conflicts of interest.

Author contributions

This work was conceived by all authors. Field work was done by JTJ. Laboratory work was done by KSL. Analysis was carried out by KSL, BPH and JBX. The manuscript was written by KSL, YQL, NZJ, BQX, and LPZ.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.104.

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